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Formulation and Evaluation of Besifloxacin Loaded In Situ Gel For Ophthalmic Delivery

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Abstract

The aim of study was to develop in situ gel of Besifloxacin by using blend polymer of sodium alginate, ethylcellulose and Xanthan gum to increase the pre-corneal residence time and better bioavailability of drug. In situ ocular gels of Besifloxacin (F1 to F6) were prepared by using polymers xanthan gum, Ethyl cellulose and sodium alginate in different ratio. The formulations F1 to F6 were evaluated for Clarity, visual appearance, pH, gelling capacity, drug content, assessment of drug release and ocular irritancy. The F1 to F6 were transparent and clear, and possessed a satisfactory gelling capacity. The drug content capacity for F1 to F6 ranged between 96.24% to 98.63%. The *in vitro* releases of drug from in situ ocular gel demonstrated that F3 (98.67%) has maximum drug release for 8 hrs compared to other formulations, and showed sustained release. The ocular irritancy study of F3 formulation showed non-irritant and safe to use. The studies suggested that prepared in situ ophthalmic gel of Besifloxacin will be an alternative for conventional eye drops and valuable alternative to counter the precorneal loss.

1 Introduction

Ophthalmic preparations are defined in the USP as “sterile dosage forms, essentially free from foreign particles, suitably compounded and packed for instillation into the eye.” In eye drug is administered at various site such as cornea, conjunctiva and sclera for better achievement of bioavailability and required effects related with the therapy. The drugs for allergies, glaucoma, bacterial infections, conjunctivitis, keratitis, local anaesthetics and viral infection can be administered at suitable sites in the eye[1].

The eye drops have very poor bioavailability due to their rapid washout during lachrymation in eyes. Most of the systems are applied as solution or suspension. The rapid pre-corneal elimination observed with conventional ocular formulation ends in poor drug bioavailability. Ease of administration in case of highly viscous solution and gel forms retard its use and patient compliance. The blurred vision and the lacharymation are associated with the dosage form involving hydrogel[2,3].

So these may be overcome by fabricating the drug as a formulation that undergoes instantaneous in situ gel formation upon ophthalmic administration. They undergo gelation after instillation due to physicochemical changes occurring in the eye. It increases the pre-corneal residence time and better bioavailability of drug can be achieved by formulating in situ gel.

Besifloxacin inhibits both bacterial DNA gyrase (topoisomerase II) and topoisomerase IV, in contrast to the older fluoroquinolones, which bind more strongly to one of the enzymes. This may minimize resistance because mutations of both enzymes are required for resistance to develop.

Besifloxacin ophthalmic solution (Besivance) is indicated for bacterial conjunctivitis caused by susceptible isolates4-6. The aim of study was to develop in situ gel of Besifloxacin by using blend polymer of sodium alginate, ethylcellulose and Xanthan gum. In situ gel solution increases the residence time and also sustain the release mechanism of the drug.

2 Material and Methods

2.1 Preparation of in situ ocular gel of Besifloxacin

The polymeric solution was prepared by dispersing required quantity of sodium alginate as main polymer and Xanthan gum, Ethyl cellulose as co-polymers in water using a magnetic stirrer until the polymers completely dissolve. Aqueous solution of Besifloxacin was added in to the polymeric solution with continuous stirring. Buffering and osmolality agents were added to the resulting solution along with benzalkonium chloride. The pH of the solution was adjusted to 6.5 using 0.1 N NaoH/0.1 N HCl. The in situ gel formulations are depicted in Table 1[7-9].

Sodium alginate, an ophthalmic gel forming mucoadhesive polymer was chosen, as the polymer; and Xanthan gum and Ethyl cellulose as copolymer. The compositions of ingredient of in situ ocular gel are shown in table 1.

Table 1: Quantity of raw materials for preparation of in situ ocular gel

Ingredient (mg)	F1	F2	F3	F4	F5	F6
Besifloxacin	500	500	500	500	500	500
Sodium alginate	400	800	1200	400	800	1200
Xanthan gum	1000	800	600	400	200	-
Ethyl cellulose	-	200	400	600	800	1000
Benzalkonium chloride (%w/v)	0.02	0.02	0.02	0.02	0.02	0.02
Sodium Chloride	700	700	700	700	700	700
Distilled water	qs	qs	qs	qs	qs	qs

2.2 Evaluations

2.2.1 Clarity and visual appearance

The clarity and visual appearance of the formulations before and after gelling was determined by visual examination of the formulations under light alternatively against white and black backgrounds.

2.2.2 pH

The pH of each of prepared ophthalmic formulations was determined by using pH meter (equip-tronics). The pH meter was calibrated before each use with standard pH 4, 7 and 9.2 buffer solutions.

2.2.3 Gelling capacity

The gelling capacity of the prepared formulation was determined by placing a drop of the formulation in a vial containing 2 ml of freshly prepared simulated tear fluid and visually observed. The time taken for its gelling was noted

2.2.4 Drug Content

The drug content estimation was carried out by diluting 1 ml of prepared formulation in 100 ml of distilled water and analyzed using UV-visible spectrophotometer (Shimadzu UV-1700 PC, Shimadzu Corporation, Japan) at 285 nm.

2.2.5 In-vitro dissolution study

The *in vitro* release of Besifloxacin from the prepared formulations was studied using a modified diffusion testing apparatus. The freshly prepared simulated tear fluid (pH 7.4) was used as a diffusion medium. Semi permeable membrane, previously soaked in the diffusion medium for overnight, was tied to one end of a specially designed glass cylinder (open at both ends) having inner diameter of 3.4 cm. Two milliliter of formulation was accurately pipette into the glass cylinder known as donor chamber.

The cylinder was suspended in a beaker (Acceptor chamber) containing 100 ml of diffusion medium so that the membrane just touches the surface of the medium. Acceptor chamber was maintained at a temperature of 37 ± 2°C with a stirring rate of 50 rpm using magnetic stirrer. About 1 ml of sample was withdrawn at a time interval of 1 hour and replaced with an equal volume of fresh diffusion medium. The aliquots were diluted with the diffusion medium and analyzed at 285 nm using UV spectrophotometer[10-14].

2.2.6 Ocular irritancy

Ocular irritation study was performed on optimized formulation in four albino rabbits (male), each weighing about 2 to 3 kg, and 0.1 ml of the optimized sterile Besifloxacin formulation was instilled in to cul-de-sac twice a day for a period of 14 days. The rabbits were monitored periodically for redness, swelling, watering of the eye[15-17].

3 Results and Discussions

In the present investigation, efforts were made to prepare the sustained release Besifloxacin in situ gel forming ophthalmic solution using polymers xanthan gum, Ethyl cellulose and sodium alginate in different ratio. The six different formulations

(F1 to F6) were prepared, and investigated for clarity, visual appearance, pH, gelling capacity, drug content, assessment of drug release and ocular irritancy.

3.1 Visual appearance

During preparation of *in-situ* ocular gel of Besifloxacin drug, visual appearance of formulation on varying the concentration of polymer in drug has been observed. The visual appearance of Besifloxacin ocular gel is displayed in table 2. The visual appearance of various formulations was transparent. It depicted that the uniformly distribution of drug in formulation.

Table 2: Visual appearance analysis of formulations

Formulations	Visual appearance
F1	Transparent
F2	Transparent
F3	Transparent
F4	Transparent
F5	Transparent
F6	Transparent

3.2 Clarity

During preparation of *in-situ* ocular gel of Besifloxacin drug, clarity of formulation on varying the concentration of polymer in drug has been observed. The clarity of Besifloxacin ocular gel is displayed in table 3. The clarity of various formulations was transparent. It depicted that the uniformly distribution of drug in formulation.

Table 3: Clarity analysis of formulations

Formulations	Clarity
F1	Clear
F2	Clear
F3	Clear
F4	Clear
F5	Clear
F6	Clear

3.3 pH

The pH of the various formulations observed and is displayed in table 4. The pH of the formulation was found to be in range of 6.05±0.82 – 6.71±0.59 which is good for eye. All the formulations of ocular gel were shown pH nearer to eyes.

Table 4: pH analysis of formulations

Formulations	pH
F1	6.05±0.82
F2	6.41±0.34
F3	6.38±0.93
F4	6.23±0.27
F5	6.71±0.59
F6	6.68±0.42

Values are mean ± S.D

3.4 Drug content

This was measured for formulations F1 to F6 in triplicate, and results are illustrated in table 5 and Fig 1. The drug content capacity for F1 to F6 ranged between 96.24±0.62% to 98.63±0.73%. The F6 revealed lowest drug content capacity while F3 displayed highest drug content capacity; and it was the highest drug content capacity compared to other formulations.

Table 5: Drug content of formulations

Formulation	Drug content (%)
F1	98.21±0.25
F2	96.24±0.62
F3	98.63±0.73
F4	97.13±0.94
F5	97.86±0.48
F6	96.32±0.62

Values are mean ± S.D

3.5 Gelling capacity

The gelling capacity was measured for formulations F1to F6 in triplicate, and results are illustrated in table 6. The gelling capacity of the prepared formulation was determined by placing a drop of the formulation in a vial containing 2 ml of freshly prepared simulated tear fluid and visually observed.

Table 6: Gelling capacity of ocular gel

Formulation	Gelling capacity
F1	++
F2	++
F3	++
F4	++
F5	++
F6	++

3.6 Assesment of in vitro drug release

The *in vitro* releases of drug from in situ ocular gel are illustrated in Fig 1. The findings of *in vitro* releases exhibited that percentage release of the drug from the developed formulations F1 (98.24%), F2 (91.24%), F5 (97.39%), and F6 (89.28%) at 6 hrs. Further the percentage release of the drug from the F3 (98.67%) and F4 (92.72%) at 8 hrs. The formulation F3 has maximum drug release as compared to other formulations, and showed sustained release. This could be the reason of higher concentration of Sodium alginate and ethyl cellulose among the developed formulations. The polymer, Sodium alginate, which undergoes instantaneous gel formation due to formation of calcium alginate by virtue of its interaction with divalent cation (Ca^{2+}) present in lachrymal fluid (pH 7.4). Alginate can be ionically crosslinked in the presence of divalent cations. Hence, F3 formulation was selected for further study. Thus the *in vitro* dissolution test indicated the sustained release nature of in situ gel of Besifloxacin.

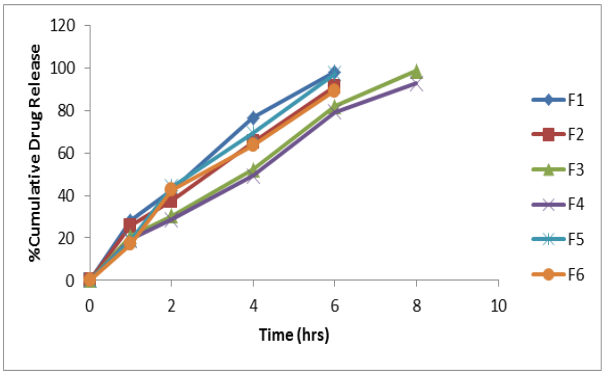

Fig 1: Comparative *in vitro* dissolution profile of various formulation of Besifloxacin ocular gel

3.7 Ocular irritation

Ocular irritation study was performed using healthy albino rabbits after getting prior permission from the institutional animal ethics committee. The eyes of each rabbits were examined at particular time interval after instillation of the optimized formulation (F3). There was no redness, continuous blinking, swelling or watering of eyes. No ocular damage or abnormal clinical signs to the cornea, iris or conjunctiva were visible. The result of ocular irritation studies indicates that formulations containing all ingredients are non-irritant to rabbit eye.

4 Conclusion

In situ ocular gels of Besifloxacin were prepared by using polymers xanthan gum, Ethyl cellulose and sodium alginate in different ratio. The formulations (F1 to F6) were evaluated on the basis of Pharmacopoeial specification. The findings of clarity, visual appearance, pH, gelling capacity, drug content, assessment of drug release and ocular irritancy were found to be complied with the Pharmacopoeial specification. The formulation F3 shows good result as compared to other formulation. Physicochemical characterization and *in vitro* drug release studies indicated that the developed formulation (F3) may prove to be a viable alternative to conventional eye drops and ointment in terms of ease of administration with added benefits of sustained drug release which may ultimately result into improved patient compliance.

5 Conflict of interests

None

6 Authors contributions

SK and PG designed the study and carried out experimental work. DJ critically revised the manuscript. All authors read and approved the final manuscript.

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